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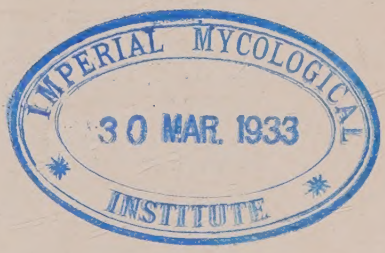
A MICROSCOPIC STUDY OF CERTAIN CHANGES IN THE MICROFLORA OF SOIL

H. J. CONN



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TECHNICAL BULLETIN No. 204

A MICROSCOPIC STUDY OF CERTAIN CHANGES IN THE MICROFLORA OF SOIL

H. J. CONN

ABSTRACT

A slight modification has been made of the method recently proposed by Cholodny for the direct microscopic study of the soil flora. This method consists essentially in leaving a microscopic slide for several days or a few weeks lying against a cut surface of soil and then staining the film of micro-organisms that has attached itself to the slide. The only important modification made in the present work is to keep the soil in jelly tumblers under controlled conditions instead of inserting the slide directly in field soil.

The method proves particularly valuable for observing changes in the soil flora from fungi or Actinomycetes to bacteria or *vice versa*.

The use of the method on an acid soil of high colloidal content indicates that the natural flora of fungi and Actinomycetes is more quickly changed to one in which bacteria predominate by increasing the moisture content than by addition of calcium carbonate. The moisture content in this particular soil at which bacteria predominate over the filamentous forms is higher than that which normally occurs in the field during the growing season of plants.

INTRODUCTION

One of the chief difficulties with which soil bacteriology has always been confronted is that ordinary bacterial methods of study do not bring out sharp differences in the flora. This was pointed out by the writer (1913)[1] about 20 years ago and there has never been any reason for modifying this conclusion so far as it applies to the ordinary culture plate method of studying the bacteria in soil.

It is, in fact, a striking thing that when the bacteria of other natural substances, such as milk or water, are examined, the numbers and kinds may vary greatly with conditions, while, on the other

[1]Refers to Literature Cited, page 20.

hand, similar methods for the study of bacteria in surface soil under conditions of moderate rainfall fail to bring out appreciable variations, either qualitative or quantitative. The number of bacteria shown by the plate method may be as low as a few million per gram or sometimes as high as 50 million, but only distinctly abnormal conditions show increases or decreases outside of these limits. Limits such as these, however, are very narrow, considering that the bacterial flora of milk may vary from a few hundred to several billion per gram. Qualitatively, also, the differences are not great. If the medium used in plating is not varied, practically the same kinds of bacteria are obtained from almost any soil, and there is very little alteration in the kinds observed unless the soil is furnished with some distinctly abnormal amount of organic matter or other bacterial nutrient.

There has been a suspicion for some time that such observations as these do not mean that the soil flora is absolutely constant, but rather that each particular medium used furnishes favorable conditions for such a small fraction of the total flora that plating on this medium gives no real indication of the variation in types or numbers of micro-organisms present. A method capable of bringing out such variations is, therefore, much to be desired.

In spite of negative conclusions such as those mentioned above, it has been realized, nevertheless, that some soils were more favorable to the growth of fungi, others to the growth of bacteria. This has been brought out by plating on media adapted respectively to the growth of these two kinds of micro-organisms. The plate method, however, has been shown to be very inaccurate as a means of judging the activity of fungi in soil. A plate count of fungi is really a count of fungal spores and it is a well-known fact that spore production may be increased when the actual growth of the fungi is decreased.

MICROSCOPIC TECHNIC

These shortcomings of the plate technic have raised a hope that the microscopic method might reveal differences in the flora not observed by the earlier procedures. Until recently, however, this hope has not been realized. The ordinary method of making microscopic preparations from soil does not permit good observation of fungus filaments and the bacteria in general all look so much alike that few differences of any significance can be observed.

The change in the situation at the present time has been brought about by a recent paper by Cholodny (1930), the full significance of which does not yet seem to be appreciated by soil microbiologists. This paper presents a new procedure for the direct microscopic study of the soil microflora and introduces an original and very simple principle of technic. This new idea was to incubate the slide in the soil, instead of transferring the soil organisms to the slide by drying soil infusion on it or otherwise. The method promises to give an entirely new conception of soil micro-organisms in their mutual relationships and to offer an interesting method of attack in studying floral changes.

Cholodny's paper seems so important for the purpose of the study undertaken in the present work that a historical discussion of the microscopic technic seems called for here to show the relationship which Cholodny's procedure bears to other similar methods.

HISTORICAL

The first suggestion of a direct microscopic study of soil microorganisms seems to have been made by the writer (1917) in a paper before the Society of American Bacteriologists. This was shortly followed by a more detailed discussion of the procedure and its application (Conn, 1918). The procedure given then has been slightly modified (Conn, 1926, 1928).

The method is essentially to make an infusion of soil (1 to 9) in dilute gelatin (0.015 per cent), to dry this on a slide, and to stain with phenolic rose bengal. This dye was selected as giving the desired degree of intensity, that is, staining the bacteria sufficiently for easy recognition, without coloring the dead organic matter too deeply. The later papers mentioned above show that other dyes of the same group, such as erythrosin or phloxine (cyanosin), can also be used with practically as good results. The writer continues to prefer rose bengal on account of its shade, but has no objection to erythrosin, which has been preferred by Winogradski and Rossi, or to phloxine.

No serious attention was given to the method by other bacteriologists, nor was it developed further by the writer, until Winogradski (1924A, 1924B, 1925) in a series of papers discussed the direct method of studying bacteria in soil. Much of Winogradski's procedure is cultural and should hardly be called a "direct method";

but part of his technic is a modification of the writer's microscopic method. The most important modification consists of the use of a centrifuge to obtain several fractions of the soil infusion, in one of which the desired micro-organisms are concentrated. Altho this modification is valuable in concentrating organisms that occur in small numbers only, it makes the method less "direct" than that originally proposed by the writer. The only other significant modification by Winogradski was to employ erythrosin instead of rose bengal, a change which is of little significance, as explained above. Erythrosin is possibly to be preferred to rose bengal in soils of high humus content, but with the ordinary agricultural soils of the United States the writer prefers rose bengal. Winogradski employed this microscopic technic primarily in studying the occurrence of *Azotobacter* in soils. In his later work, in which he has developed methods for studying the distribution of other kinds of bacteria in soil, he has preferred the use of silicate jelly plates rather than the microscopic technic; accordingly, the latter has not been further developed by him.

Meanwhile, Winogradski's endorsement of the microscopic procedure has brought it to the attention of various European soil bacteriologists, some of whom even seem to be unaware that it did not originate with him—altho Winogradski makes proper acknowledgment as to its authorship. Several of these later investigators have made important modifications of the procedure. Notable among these are the contributions of Koffman (1928, 1931), Rossi (1928), Rossi and Riccardo (1927), Rossi and Gesuè (1930), as well as Cholodny (1930).

Koffman's work is intended less to develop a direct method than to adapt the microscopic technic for accurate counting. As stated above, the usual microscopic technic has been criticized because accurate counts cannot be obtained by it. Koffman, on the other hand, has devised quite an elaborate apparatus for agitating and sampling soil infusion and has worked out methods for fixing and staining an accurately measured portion of the infusion without drying it to the slide. By means of this method none of the soil particles or micro-organisms are lost so that a quantitative determination of the different kinds of micro-organisms seen can be made.

Also of interest in connection with Koffman's paper is the dye which he employs. He calls the dye "alcohol-soluble cyanosin" and very commendably gives its formula so that one can tell just what

he is using. The dye is the ethyl ester of cyanosin or phloxine. This is a dye with which the writer has had no experience. It should bear the same relation to phloxine that alcohol-soluble eosin does to eosin Y. This dye certainly deserves study as a stain for bacteria in soil.

Rossi's procedure is entirely different and is, beyond question, the most direct method for examining bacteria in the soil that has been proposed. A vertical surface of soil is exposed and a slide held in a special holder is pressed against the surface. The slide is then removed and the contact preparation thus obtained is stained with erythrosin. This method shows the colonies of micro-organisms as they actually occur on the soil and brings out many points of interest. It has not yet been much employed elsewhere, partly because of the difficulty of obtaining a holder for the slides such as that described by Rossi. The modification of the microscopic method which is being considered in the present paper was proposed a few years ago by Cholodny (1930). The procedure as described by its originator is to cut a shallow trench in the soil and then to press a slide against the vertical surface of the undisturbed soil with one end of the slide a little above the top of the soil. The method differs from that of Rossi, however, in that the slide is not removed immediately but that the soil is packed in loosely behind it and the slide allowed to stay in place for some time. When it is finally taken out, the soil is removed carefully behind the slide and the latter pulled away sharply from the undisturbed soil so as to leave the film attached to the slide which has been formed by the micro-organisms growing upon it. This slide is dried and stained.

FIG. 1.—A TUMBLER OF SOIL WITH TWO SLIDES INSERTED FOR STUDYING THE FLORA BY THE CHOLODNY METHOD.

EXPERIMENTAL

METHODS EMPLOYED

The procedure adapted in the present work is that of Cholodny with only very minor modifications. Instead of digging a trench in the field soil for inserting the slide, the soil is placed in jelly tumblers provided with a cover, and two slides are inserted. (See Fig. 1.) Altho soil thus handled is likely to be quite different from that in the field itself, this modification gives the advantage of making it possible to control the conditions and to investigate the effect of various treatments on the soil to much better advantage than can be done in the field.

FIG. 2.—A COLONY OF BACTERIA, A SMALL ACTINOMYCES COLONY, AND A FUNGUS FILAMENT AS SHOWN BY THE CHOLODNY TECHNIC.

A second slight modification is in the time of incubation. Cholodny recommended incubation for about 2 weeks before removing the slide, while the writer has ordinarily found more interesting results if the slides are kept in place only about 5 to 7 days. Actually, the time of incubation has varied. Usually one slide is removed in about 5 days and a new one put in its place. The second slide is removed in about 7 days from the time of insertion, and the slide in-

serted where the first was removed is taken out after being in place 5 or 6 days.

The stain employed in the present work has been rose bengal, not erythrosin as employed by Cholodny.

The formula is as follows:

Rose bengal	1	gram
Calcium chloride	0.01	gram
5 per cent aqueous phenol	100	cc

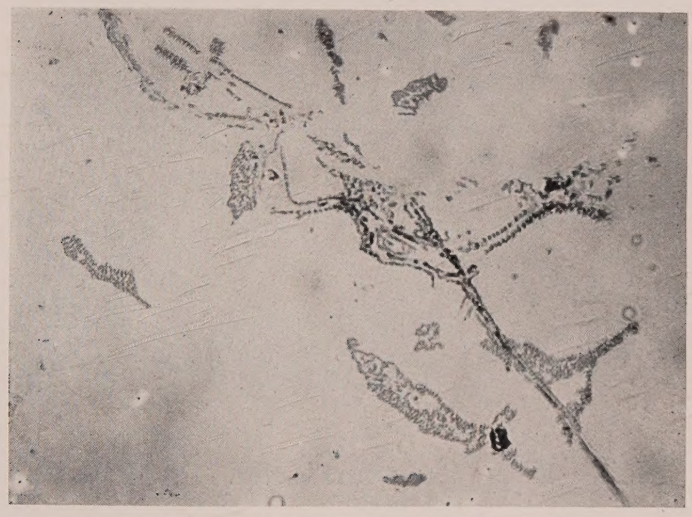

FIG. 3.—A DECAYING FUNGUS FILAMENT IN SOIL SURROUNDED BY MASSES OF BACTERIA.

Two methods of applying the stain have been tried. One method is to let the slides lie flat on a surface over a boiling water bath and to pour the stain on top of the slide allowing it to stay 1 minute, replenishing it occasionally to keep it from drying out. The second method is to keep the stain in a staining jar which sets in boiling water, then to insert the slides after the stain inside the jar begins to steam. The slides are allowed to stay in the hot staining fluid for 1 minute. The former method seems to give the better stained preparations.

USE OF THE PROCEDURE

Cholodny's paper is accompanied by a considerable number of photo-micrographs showing various types of colonies observed in the

soil by his method. Practically all of the forms photographed by him have been observed here (Figs. 2 to 4).

Fig. 2 is interesting as showing a fungus filament crossing the field, an Actinomyces colony, and a colony of bacteria. Fig. 3 shows

FIG. 4.—AN ACTINOMYCES COLONY IN SOIL.
The Cholodny method furnishes a picture of conidiophores of both Actinomycetes and fungi in their natural orientation, thus giving some clue as to identity of species.

a fungus filament surrounded with bacteria. This phenomenon is shown by Cholodny in his Figs. 8 and 9 and has been frequently observed here. It seems to be of some significance in soil, but its exact meaning is not yet determined. The bacteria surrounding these mold filaments might be merely physically attached, but the frequency with which such filaments are found to be devoid of stainable contents suggests that they are dead and that the bacteria are living upon the organic matter of which they are composed. Indications are at hand that this phenomenon is likely to be noticed when a predominantly fungal flora is being altered to a bacterial flora in some soil.

The method is being used in the writer's laboratory for three different purposes, for all of which it appears to have considerable promise, as follows: First, in determining differences in the flora of

different soils; second, in determining the effect of fertilization and moisture changes on the flora; and third, in studying colony production in pure culture.

The first of these three purposes, that is, studying differences in the flora of different soils, is one of the lines in which this procedure seems most interesting. This is the case because it is quite difficult by many bacterial methods to bring out the distinct differences between different soils. The Cholodny method, on the other hand, shows very distinct differences. Under some conditions the flora appears to be predominantly fungal; under others bacterial. These distinctions show up by this technic even tho the plate method and the usual microscopic technic show no appreciable differences. It is still uncertain, to be sure, whether they represent inherent differences between the soils or merely those due to treatment. Altho this point still remains to be determined, the great differences that have been observed are very significant in view of the lack of variation observed by other methods.

The second application of the method which seems to be important is in studying the effect of soil treatment upon the flora. This is difficult to undertake by the procedure originally described by Cholodny but is easy to accomplish when the method is modified by the use of tumblers as here suggested. It has already proved possible by the addition of mineral salts and by changes in moisture content to alter the flora of a soil from one type to another, and such changes are brought out very distinctly by this procedure. In the writer's opinion this is at present one of the most promising uses to which the method can be put.

Whether the procedure be employed for studying differences between soils or effects of treatment of a soil, one defect of the method must be taken into account, namely, that there is no way of securing a representative sample to examine. There is evidence that the picture may be distinctly different on two slides a short distance apart. One must, therefore, examine several slides from the same soil under identical conditions before drawing definite conclusions. It still remains to be determined how serious this weakness of the method is in practical use.

Another weakness of the technic at the present time is the inability to judge the species of the different forms observed. This is particularly true in the case of bacteria with a very slight morphological

variation. Molds and Actinomycetes, on the other hand, may well prove much easier to separate one from another. One of the strong points of the procedure is that the different organisms occur in their natural orientation. In the case of the two groups of organisms just mentioned, this is particularly important because the technic shows the arrangement of spores and in this way may give an actual clue as to the identity of species. No such definite information can be obtained in the case of bacteria, altho it is noticeable that they occur in characteristic colonies. This is the reason for the third application of the method suggested above, namely, the study of colony production in pure culture.

The exact technic for this pure culture work has not yet been perfected. It is, of course, necessary to sterilize the soil, to inoculate it, and to maintain it free from contamination. This can sometimes be done in tumblers by keeping their tops wrapped in cotton. The method is awkward and not always efficient, however, and it is hoped that it can be improved upon. There seems to be no reason why large test tubes and unusually narrow slides cannot be used for the purpose. Little has as yet been done here by the study of pure cultures by this method and no knowledge of their natural colony forms that is at all conclusive has been obtained. The idea is merely offered here as a suggestion of a possible valuable application of the procedure.

THE SOILS STUDIED

The soils used in this work were the same four that were under investigation in certain earlier work (Conn and Darrow, 1930). Reference is made to this earlier work for a detailed description of the soils, and they will be described here briefly.

Volusia silt loam.—A hill soil low in calcium and acid in reaction (pH 4.4), containing a rather large percentage of colloids, the ratio of colloids to calcium being over 100:1. The sample employed in the present work was from the same field east of Canandaigua Lake as the sample reported in the previous bulletin.

Hoosick coarse sandy loam.—A sample from a field near Kinderhook, N. Y., that had been undisturbed for years. This soil is as acid as the Volusia soil and with about the same calcium content. The percentage of colloids is considerably lower than the Volusia soils, the ratio of the colloids to calcium being 60:1.

Dunkirk silty clay loam.—A sample from one of the Station fields at Geneva of a soil about neutral in reaction, with a fairly high calcium content. The percentage of colloids is about the same as in the case of the Hoosick soil, the ratio of colloids to calcium being 35:1.

Dunkirk fine sand.—A soil from north of Geneva about neutral in reaction, but too sandy and devoid of organic matter to yield good crops. It is extremely low in colloidal material, the ratio of the latter to calcium being only about 8:1. This soil has a very high carbon nitrogen ratio (20:1).

TESTS COMPARING THE FOUR SOILS

It was realized from the start that to make a fair comparison between these four soils, two of which are distinctly acid, it might be necessary to add some carbonate to adjust the reaction. This can readily be accomplished by adding 1 per cent of calcium carbonate to each tumbler of soil. With this amount of carbonate, the reaction of each soil is brought to about pH 6.6.

In the first experiments which were set up, calcium carbonate was ordinarily omitted, and each soil without addition was compared with the same soil containing also 1 per cent of some potassium or sodium salt. The combinations of salts employed are shown in Table 1 in which the results of the tests are also given. The chief reason for adding a potassium or sodium salt was to furnish each soil with potassium and phosphorus separately and in combination to see whether any change in flora produced would correlate with the addition of either of these fertilizer ingredients. A further object in adding these salts was to see if any observations could be made similar to those reported in Technical Bulletin No. 172 of this Station, namely, that the bacteria studied were stimulated in the two acid soils by the addition of some one of these salts.

The Cholodny method does not permit quantitative measurements. It is possible, however, to make rough estimates as to the abundance of the various types of organisms observed. To indicate this in the tables of this bulletin, each group of micro-organisms recognized by its appearance under the microscope is designated by a letter and its abundance indicated by differences in the type used, the significance of the different letters and type faces being indicated in footnotes to the tables.

TABLE 1.—TYPE OF FLORA SHOWN BY CHOLODNY METHOD IN FOUR SOILS WITH VARIOUS ADDITIONS.

SOIL AND DATE OF BEGINNING OF EXPERIMENT	TUMBLER NO.	INGREDIENTS ADDED TO SOIL	TYPES OF ORGANISMS SHOWN ON SLIDE IN SOIL*		
			Dec. 2 to 7	Dec. 2 to 9	Dec. 7 to 15
Dunkirk silty clay loam, Dec. 2, 1931	1	Nothing	**A F** s	s *c l F*	**s c** A F
	2	1% K_2CO_3	**c F** *s*	s *c A*	s c *A F*
	3	1% K_2SO_4	**s** A F	s c *A*	s c *A F*
	4	1% K_2HPO_4	**s** A *F*	A s l F	**s c** l A F
	5	1% Na_2HPO_4	**s** A *F*	A c s F	**s c** A
	6	1% Na_2HPO_4+1% K_2SO_4	**s** A *c*	**s A F** c	**s c** A
			Dec. 18 to 23	Dec. 18 to 26	Dec. 23 to Jan. 6
Dunkirk silty clay loam, Dec. 17, 1931	1	Nothing	**s A**	A F	**c s** A *F*
	2	1% K_2CO_3	s l A F	**A F** s *o*	**c s** A F
	3	1% K_2SO_4	**A** c s *F*	**c A F**	**c A** s F
	4	1% K_2HPO_4	c	**c A F** *s*	s c F *A*
	5	1% Na_2HPO_4	**A** c	**c A**	**s A** c F
	6	1% Na_2PHO_4+1% K_2SO_4	**c A**	. . . .	**A** c s F
			Jan. 19 to 25	Jan. 19 to 27	Jan 22 to Feb. 1
Dunkirk fine sand, Jan. 18, 1932	1	Nothing	*c s*	**c s** *o S A*	c s *S F*
	2	1% K_2CO_3	**s** l *F*	s l S *c*	c s l *A F*
	3	1% K_2SO_4	c s	c s *L A*	c s S
	4	1% K_2HPO_4	**s** c L S	**c s** *A F*	**c s**
	5	1% Na_2HPO_4	s *c S*	**s** c *S*	**c** s *F*
	6	1% Na_2HPO_4+1% K_2SO_4	**s** c S	c s A *L*	**c** A *F*
			Jan. 29 to Feb. 6	Jan. 29 to Feb. 9	Feb. 6 to 15
Hoosick coarse sandy loam, Jan. 28, 1932	1	Nothing	c F	A s F	**F A** s
	2	1% K_2CO_3	s A F *c o*	s A F *c S*	**A F** *c s S*
	3	1% K_2SO_4	c A F	**F** *c A*	A F *s*
	4	1% K_2HPO_4	**A F** s *c S*	c A	**c s** A *S*
	5	1% Na_2HPO_4	**F** *s c S A*	s c A F	**c A** s F *S*
	6	1% Na_2HPO_4+1% K_2SO_4	**F A** *s*	**F A** *s c*	**A** c F
			Jan. 19 to 25	Jan. 19 to 27	Jan. 22 to Feb. 1
Volusia silt loam, Jan. 18, 1932	1	Nothing	**c s F**	**F** c *A*	F *c l*
	2	1% K_2CO_3	**F** c	**A F** s	**F** s *c A*
	3	1% K_2SO_4	**F** *c s A*	**F** s A	**F** *A*
	4	1% K_2HPO_4	**s F** c	**F** c s *A*	**F** c s A
	5	1% Na_2HPO_4	**F** c s	**F** c	**s F** c
	6	1% Na_2HPO_4+1% K_2SO_4	**F** A	s c A F	A s F *c*
			Jan. 29 to Feb. 6	Jan 29 to Feb. 9	Feb. 6 to 15
Volusia silt loam, Jan .28, 1932	1	1% $CaCO_3$	**s F** c	F *c s*	**s F** c
	2	1% $CaCO_3$+1% K_2CO_3	**s A F** c *o*	**F** L S	**s A F** c
	3	1% $CaCO_3$+1% K_2SO_4	**c F** s A	**F** *c*	**F** s
	4	1% $CaCO_3$+1% K_2HPO_4	**F** c s *A*	c s A F	**c s A F**
	5	1% $CaCO_3$+1% Na_2HPO_4	**c F** s A *S*	**c F** s A	**c A** s F
	6	1% $CaCO_3$+ 1% Na_2HPO_4+1% K_2SO_4	**F** c s	**F** c s	**F** *c s*

*The letters in these columns indicate the following groups of organisms:

c=coccoid forms
s=short rods
l=long, slender rods
o=oval forms
L=large rods (probably spore-formers)
S=bacterial spores
A=Actinomycetes
F=fungi

Abundance is indicated by the type face as follows: Very abundant, **bold face**; moderately abundant, roman; few, *italics.*

One point of very decided significance brought out by Table 1 is that the natural flora of the Volusia soil is primarily fungal, while that of the two Dunkirk soils is predominantly bacterial. It was to be expected, of course, that an acid soil would favor fungi more than a neutral soil, but it was rather striking that the addition of calcium carbonate (see test of January 28) corrected the reaction of the Volusia soil without greatly changing its flora. One would hardly suppose that the original reaction of the soil could continue to affect the type of organisms growing therein for about 2 weeks after adding the carbonate.

This observation was considered to indicate that when a fungus flora is once established in the soil it continues to predominate over the bacteria for some time after making the reaction more favorable to the latter organisms. It was thought that some other factor in addition to the correction of the reaction might possibly help to bring about a change in the flora, allowing the bacteria to become established.

The above data were examined with this thought in mind and they seem to indicate a slight tendency for the bacteria to predominate over the molds where phosphate was added. This was particularly noticeable in the experiment of January 28. In general, however, the differences produced by the addition of any of the salts employed in these tests were not sufficiently noticeable to be regarded as significant.

VARYING THE MOISTURE CONTENT OF THE VOLUSIA SOIL

In considering the possible reason why bacteria did not become predominant in the Volusia soil, even after lime had been added, it was thought that this might be due to the fact that they were entirely absent in this soil and could not be made to develop unless inoculated. Inoculation was attempted by adding 1 per cent of the Dunkirk silty clay loam (in which bacteria usually predominate) to the soil of the experiment of January 18 and January 28, after making the readings given in Table 1. At the same time this was done more water was added to the soil to restore the original moisture content. By mistake, considerably more water was added than was originally present.

The results of this experiment, as shown in Table 2, indicate a decided change in the soil flora from molds to bacteria. It was thought

at first that this must be the result of the inoculation, but on further thought the increased moisture content was considered an equally possible explanation.

TABLE 2.—TYPE OF FLORA SHOWN BY CHOLODNY METHOD IN VOLUSIA SILT LOAM AFTER ADDING 1% DUNKIRK SILTY CLAY LOAM.

HISTORY OF TUMBLERS	TUMBLER NO.	INGREDIENTS ADDED TO SOIL ON JAN. 18 AND 28, RESPECTIVELY	TYPES OF ORGANISMS SHOWN ON SLIDES IN SOIL*		
			Jan. 29 to Feb. 6	Jan. 29 to Feb. 9	Feb. 6 to 15
These tumblers originally set up Jan. 18, 1932 (Table 1). On Feb. 18, 1% Dunkirk soil and additional water were added.	1	Nothing	**F** c S	c s F	c s *F*
	2	1% K_2CO_3	c s **A**	s **A** c l	c s A *l L S*
	3	1% K_2SO_4	**F**	**A** c s F	**A** c s F
	4	1% K_2HPO_4	**F** c s	**C** s A *F*	c s l *A*
	5	1% Na_2HPO_4	c s **A F**	s c F *A*	c s A F
	6	1% Na_2HPO_4+1% K_2SO_4	c s **A** *F*	c s l *o S A*	c s A F
These tumblers originally set up Jan. 28, 1932 (Table 1). On Feb. 18, 1% Dunkirk soil and additional water were added.	1	1% $CaCO_3$	c **F** *s*	c s F	c s A F
	2	1% $CaCO_3$+1% K_2CO_3	l **A** c F	s A F	c s A F *L*
	3	1% $CaCO_3$+1% K_2SO_4	s **F** c	c s **A F**	c s **A F**
	4	1% $CaCO_3$+1% K_2HPO_4	s **A** *c*	**A F** *c s*	s c A F *L*
	5	1% $CaCO_3$+1% Na_2HPO_4	c s **A F**	c **A F** s	**F** c
	6	1% $CaCO_3$+ 1% Na_2HPO_4+1% K_2SO_4	**A F** *s*	s c A F	**F** c

*The letters in these columns indicate the following groups of organisms:
c=coccoid forms
s=short rods
l=long, slender rods
o=oval forms
L=large rods (probably spore-formers)
S=bacterial spores
A=Actinomycetes
F=fungi
Abundance is indicated by the type face as follows: Very abundant, **bold face**; moderately abundant, roman; few, *italics*.

To test out this point, the experiment given in Table 3 was planned. In this work, different lots of the soil were adjusted to different moisture contents and some were inoculated with 1 per cent of the Dunkirk silty loam, while others were not. The results of this experiment show that a change from a fungal to a bacterial flora did occur in some tumblers, but that these were instances of high moisture content rather than where an attempt to inoculate with the other soil had been made.

In fact, the work reported in Table 3 seems to indicate that moisture content is of more importance than reaction in changing the

TABLE 3.—TYPE OF FLORA SHOWN BY CHOLODNY METHOD IN VOLUSIA SILT LOAM OF TWO DIFFERENT MOISTURE CONTENTS WITH AND WITHOUT ADDITION OF 1 PER CENT DUNKIRK SILTY CLAY LOAM.*

HISTORY OF TUMBLERS	TUMBLER NO.	ORIGINAL MOISTURE CONTENT, PER CENT	MOISTURE AFTER MAR. 23, PER CENT	INGREDIENTS ADDED TO SOIL	TYPES OF ORGANISMS SHOWN ON SLIDE IN SOIL†					
					No $CaCO_3$ present			After adding 1% $CaCO_3$ on Apr. 7		
					Mar. 15 to 23	Mar. 15 to 30	Mar. 23 to Apr. 2	Apr. 7 to 13	Apr. 7 to 18	Apr. 13 to 21
No Dunkirk soil added to these tumblers	1	8.4	13.9	1% $CaCO_3$	**F** A	A F *s o*	s A	**A F** *c*	A F	**F** *c s A*
	2	8.4	13.9	1% $CaCO_3$	F *A*	*c s*	c s A F	c‡ A F	F *c s A*	**F** c A *s*
	3	8.4	13.9	1% $CaCO_3$+ 1% K_2CO_3	**F** *A*	A *c s F*	A c s F ‡	**c s A** *l*	**A** s	c s A *F*
	4	8.4	13.9	1% $CaCO_3$+ 1% K_2CO_3	A F	**c s**‡ A F	‡ A *s*	**c A** *s*	c s *A*	A F *c s*
	5	19.3	19.3	1% $CaCO_3$	F *A*	**c s A** F	**F** c s	**c** s F	c s *o L S A F*	A F
	6	19.3	19.3	1% $CaCO_3$+ 1% K_2CO_3	**A F** *s*	c s A	s *c A F*	c s *F*	c s *A F*	**c s A** *o L S F*
The soil in these tumblers mixed with 1% Dunkirk silty clay loam	7	8.4	13.9	1% $CaCO_3$	A F	s A F	*s c A F*	**A F** c	A *F*	**F** A *s*
	8	8.4	13.9	1% $CaCO_3$	A F *s*	*s l A F*	c *s l S A F*	c A F	F *c A*	**A** F
	9	8.4	13.9	1% $CaCO_3$+ 1% K_2CO_3	**A c** ‡F	**A** *l o*	**c s** *A*	**A F**	A c s F	**A** *c s*
	10	8.4	13.9	1% $CaCO_3$+ 1% K_2CO_3	A F	**s A** c *o F*	s c A F	**A** s *F*	c s A	c s A
	11	19.3	19.3	1% $CaCO_3$	s A F	s A	**s c** l A F *o*	c s	s l *L S F*	**F** s
	12	19.3	19.3	1% $CaCO_3$+ 1% K_2CO_3	s c A *F*	s c *F*	**s c** *A F*	**s c** *A F*	**s c** *F*	**c s** *F*

*Experiment set up March 15, 1932.

†The letters in these columns indicate the following groups of organisms:

c=coccoid forms
s=short rods
l=long, slender rods
o=oval forms
L=large rods (probably spore-formers)
S=bacterial spores
A=Actinomycetes
F=fungi

Abundance is indicated by the type face as follows: Very abundant, **bold face**; moderately abundant, roman; few, *italics*.

‡These coccus and short-rod forms may have been Actinomyces conidia.

flora from one in which fungi predominate to one which is principally bacterial. It will be noticed that the tumblers in this experiment were run for about 3 weeks before the lime was added. In some cases the change to a bacterial flora had occurred before the

TABLE 4.—TYPE OF FLORA SHOWN BY CHOLODNY METHOD IN VOLUSIA SILT LOAM OF VARIOUS MOISTURE CONTENTS.*

HISTORY OF TUMBLERS	TUMBLER NO.	ORIGINAL MOISTURE CONTENT, PER CENT	TYPES OF ORGANISMS† SHOWN			
			On slide in soil before drying			On slide in soil after partial drying, June 14 to 21
			May 27 to June 1	May 27 to June 4	June 1 to 11	
No Dunkirk soil added to these tumblers, but each received 1% $CaCO_3$ + 1% K_2CO_3	1	9.8	A F	A *F*	A	
	2	13.7	s A F *l*	A F *s*	A *F*	
	3	17.5	A s l L S F	A F c s *S*	A *s l F*	
	4	21.4	s A F *L S*	c s S A	c s	A
	5	25.2	c s S *A F*	s c *F*	s c F *L*	A
	6	29.1	c s o S A F	c s S	c s F	A F *c*
The soil in these tumblers mixed with 1% Dunkirk silty clay loam; each tumbler also received 1% $CaCO_3$ + 1% K_2CO_3	7	9.8	A F	A	A	
	8	13.6	s A F *L S*	F A *l*	A *S*	
	9	17.4	F s A *c*	A c s *F*	c s A *F*	
	10	21.2	c s	c s A F *S*	c s *l S A F*	A F
	11	25.0	c s S A F	c s L S *F*	s c	A s *F*
	12	28.8	s c *S F*	c s S F	c s *A*	A c F

*Experiment set up May 27, 1932.

The letters in these columns indicate the following groups of organisms:

c=coccoid forms
s=short rods
l=long, slender rods
o=oval forms
L=large rods (probably spore-formers)
S=bacterial spores
A=Actinomycetes
F=fungi

Abundance is indicated by the type face as follows: Very abundant, **bold face;** moderately abundant, roman; few, *italics.*

addition of lime, but in practically no instance did the change take place except in those tumblers which had an initial moisture content of about 20 per cent.

Another experiment to test out this same point is reported in Table 4. In this instance, both calcium carbonate and potassium carbonate were added to each tumbler. Varying amounts of water, however, were added to the different tumblers so that the moisture-

holding capacity of this soil is about 40 per cent. The tumblers with the highest moisture content in this series, altho considerably short of saturation, were nevertheless so wet that it was difficult to avoid puddling. Two tumblers were prepared of each moisture content and to one of each pair was added 1 per cent of the Dunkirk silty clay loam to see whether it had any inoculating effect. The addition of this 1 per cent of the other soil made a slight change in the moisture content as will be noticed by comparing tumbler No. 6 with No. 12 and tumbler No. 5 with No. 11, etc. For practical purposes, however, the moisture content of each of these may be regarded as the same.

After about 2 weeks, when the third slide was removed from each tumbler, the six tumblers with the highest moisture content were allowed to dry out partially. Three days later, when the moisture content had dropped to perhaps 15 per cent, another slide was inserted and left for 7 days longer.

The results reported in Table 4 show very clearly that the change to a bacterial flora occurred only in the tumblers with the highest moisture content. After partially drying, the bacteria again became scarce and the predominating organisms proved to be Actinomycetes with some fungi.

DISCUSSION

The most interesting conclusion from the work reported in this bulletin is that the Cholodny method of soil examination is satisfactory to indicate a change in the predominating flora of a soil from fungi or Actinomycetes to bacteria or *vice versa*. This method seems to be the only one so far devised that can bring out this point, since the plate method tends to count only the spores of the filamentous organisms, while the earlier microscopic methods fail to show these organisms in anything like their true abundance. It is unfortunate, to be sure, that the Cholodny method does not permit exact quantitative measurements. In spite of this fact, however, the estimates that can be made by the procedure are thought to be worth more from the above-mentioned standpoint than any which can be obtained by the plate method or the microscopic examination of the dried smears.

The use of this method in the examination of an acid soil very high in colloidal material seems to indicate that moisture content is

rather more important than reaction in producing a change in the flora of this soil from one of fungi and Actinomycetes to one of bacteria. This would suggest that the activities in this soil are mostly carried on by bacteria during periods of moderately high rainfall and that at other times the fungi and Actinomycetes are the most active organisms.

Under actual field conditions there are not many periods of any great length during the summer or early fall when the moisture content of this soil is over 15 per cent. It seems quite probable, therefore, that the filamentous organisms are the most active ones under normal conditions. Plate counts from the soil, however, hardly bring out this fact, but suggest on the contrary that the flora is quite meager. The soil is known to be one in which organic matter decomposes very slowly. It is quite possible that absence of bacterial activity, except during periods of high moisture content, may account for the slow rate of decomposition. If this is the case, one of the chief faults of the soil may be the large amount of colloidal matter it contains which absorbs the moisture content and in that way prevents it from being a sufficiently favorable medium for the growth of bacteria causing decay.

LITERATURE CITED

Cholodny, N.

1930 Ueber eine neue Methode zur Untersuchung der Bodenmikroflora. *Arch f. Mikrobiol.*, **1**, *620–652.*

Conn, H. J.

1913 The distribution of bacteria in various soil types. *Jour. Amer. Soc. Agron.*, **5**, *218–221.*

1917 The direct microscopic examination of bacteria in soil. *Abstr. Bact.*, **1**, *40.*

1918 The microscopic study of bacteria and fungi in soil. *New York Agr. Exp. Sta. Tech. Bul. No. 64.*

1926 An improved stain for bacteria in soil. *Stain Tech.*, **1**, *125–128.*

1928 On the microscopic method of studying bacteria in soil. *Soil Science*, **26**, *257–259.*

Conn, H. J., and Darrow, Mary A.

1930 Influence of various non-nitrogenous compounds on the

growth of certain bacteria in soils of low productivity. *New York State Agr. Exp. Sta. Tech. Bul. No. 172.*

Koffman, M.

1928 Ein Methode zur direkten Untersuchung der Mikrofauna und der Mikroflora des Bodens. *Centb. f. Bakt., 2 Abt.,* **75,** *28–45.*

1931 Weiteres zur Methode der direkten Untersuchung der Mikrofauna und Mikroflora des Bodens. *Centb. f. Bakt., 2 Abt.,* **85,** *1–11.*

Rossi, G. M.

1928 Il terreno agrario nella teoria e nella realta. *L'Italia Agr., No. 4, Apr. 1928.*

Rossi, G., and Gesuè, G.

1930 Di un nuovo indirizzio nella studio biologico del suolo. *Ann. Tcchnica Agraria,* **3,** *196–248.*

Rossi, G., and Riccardo, S.

1927 L'esame microscopio e bateriologico diretto del terreno agrario. *Nuov. Ann. dell Agr.,* **7,** *457–470.*

Winogradski, S.

1924A Sur l'étude microscopique du sol. *Acad. Sci., C. R.,* **179,** *367.*

1924B La methode directe dans l'étude microbiologique du sol. *Chim. et Indus.,* **2,** *No. 2, Feb. 1924.*

1925 Études sur la microbiologie du sol. *Ann. Inst. Past.,* **39,** *1–56, 299–354.*